CHARGE NUMBER: Project 1904

PROJECT TITLE: Tobacco Physiology and Biochemistry

PERIOD COVERED: October 1-31, 1985

PROJECT LEADER: I. L. Uydess

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Objective: To establish the time course and biochemical changes characteristic of tobacco leaves at various stages during senescence.

Status:

I. Immunochemical Phytohormone Assays (In collaboration with B. Davies).

Aqueous, 80% methanol extracts of greenhouse and field-grown, green leaves, and greenhouse-grown, senescing leaves of Coker 319 tobacco have been used in the initial testing of an enzyme-linked, immuno-sorbant assay ("ELISA") kit from Idetek, Inc. for abscisic acid (ABA). Although the values obtained were unexpectedly high and far above the range of the standards employed due to the inadvertent use of overly concentrated extracts (low values were initially expected), the preliminary indications are that these kits can be used successfully in the analysis of abscisic acid in our tobacco leaf samples. Similar procedures will be employed with regard to the evaluation of indole acetic acid (IAA) in these, as well as in other tobacco leaf extracts over the next two months.

II. Enzyme Assays and Associated Methodologies.

The methodology for the analysis of carboxypeptidase B using a commercial enzyme preparation has been established, and is currently being employed to evaluate carboxypeptidase B activity in tobacco leaf extracts. However, as in the cases of endopeptidase and carboxypeptidase A, no activity has been demonstrated in either frozen greenhouse or field-grown leaves, or fresh greenhouse leaves using several different extraction procedures. The leucine aminopeptidase (LAP) assay, on the other hand, continues to demonstrate the activity of this exopeptidase in all of the tobacco extracts examined. Project 1904 (and associated personnel) are currently investigating the basis for this problem along several lines including:

1. Concentration of Extracts: Is this a concentration problem with respect to an extremely low level of some of these enzymes in the mature and/or senescing leaves of the tobacco cultivar being evaluated?

Results: Two experiments were conducted in which tobacco leaf extracts were concentrated approximately 3 and 10-fold using an Amicon membrane filtration device. However, no activity was subsequently realized in either of these concentrated extracts for endopeptidase. Additional

experiments utilizing ammonium sulfate and membrane concentration procedures are in progress.

It is also possible that Coker 319 (whether field or greenhouse grown) has extremely low levels of these two groups of enzymes, or alternatively, that these particular tobacco enzymes have different specificities with regard to what is (or what is not) an acceptable substrate (the substrate specified for the commercial enzyme preparation is generally employed). To explore these two possibilities, a variety of tobacco cultivars will be examined, along with alternative substrates.

2. Protease Inhibition: Are some of these problems due to the inactivation of the targeted tobacco enzymes as a result of the use of protease inhibitors during our extraction procedures (inhibitors of protein degradation normally used to protect such enzymes)?

Results: Two experiments were conducted employing a commercially available, yeast endopeptidase (used normally as our control), as well as two plant-specific proteases (bromelain and papain) thought to be more homologous to the endogenous tobacco enzyme than that isolated from yeast. In each case, the protease inhibitors currently being used (leupeptin and PMSF) were added to one of two duplicate tubes containing one of each of the above-mentioned yeast or plant enzymes in order to assay for the possible inhibition of these plant specific enzyme activities. Leupeptin, but not PMSF, was found to inhibit the activity of the two plant proteases, while PMSF (but not leupeptin) was found to inhibit the yeast enzyme. Thus, while this question has not been completely resolved by these initial experiments, the results do suggest that further work in this area is required, and that caution must be exercised when planning to utilize such protease inhibitors in the evaluation of plant protease enzyme activity.

Plans: To continue testing for enzyme activities in tobacco extracts prepared from material grown in the greenhouse or in the field under various conditions.

—Jan Hydes

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